

EFFECT OF POSTHARVEST CALCIUM CHLORIDE VACUUM INFILTRATION ON THE SHELF LIFE AND QUALITY OF TOMATO (CV. 'THILINA')

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ABSTRACT

Mature turning tomato fruits (cv. 'Thilina') were treated with four different concentrations of CaCl₂ (0%, 2%, 4%, and 6% aqueous solutions) using different modes of application; dipping, vacuum infiltration and pressure infiltration, with a view to improve the shelf life and quality. Fruits vacuum infiltrated at -20 kPa and treated with the four different concentrations of CaCl₂ were assessed for pH, total soluble solids (TSS) content, percentage titratable acidity (%TA) and firmness. Total calcium content in the inner and outer pericarp tissues was estimated and the path of calcium infiltration was revealed using black ink. Vacuum infiltration was found to be the most effective treatment with respect to shelf life extension. Also, 6% CaCl₂ treatment at -20 kPa was the best in terms of extension of shelf life (by 92%) and in keeping the postharvest quality of tomatoes compared to the untreated fruits kept at 28 °C. Fruit firmness significantly increased with CaCl₂ application. Delay in fruit colour development, lowering of ethylene production rates and delay in the time taken to reach the ethylene climacteric were observed with increased CaCl₂ concentration. Treatment with CaCl₂ did not have a considerable effect on fruit pH, TSS or %TA. The amount of total calcium in the inner and outer pericarp regions increased significantly with calcium application. The stem end scar was found to be the main pathway of CaCl₂ infiltration in to fruits.

Key words: firmness, ethylene, ripening

INTRODUCTION

Tomato (*Lycopersicon esculentum*, Mill) is one of the most widely consumed vegetables in the world. For fruits like tomato, postharvest handling is as critical as production practices due to their delicate nature. Postharvest losses may take place at any stage in the handling system from harvesting through storage and marketing to final delivery to the consumer. Due to its climacteric nature, tomato is highly perishable especially in tropical and subtropical areas. Nearly 30-50% of the produce is lost after harvest because of inadequate handling and preservation (Inaba and Crandall, 1986).

Calcium ions are known to be involved in many physiological processes in fruits and vegetables, playing an important role in maintaining their quality. Increased Ca⁺² levels have been shown to reduce respiration and ethylene production rates in a variety of fruit crops including tomato (Garcia *et al.*, 1995). Effectiveness of the method of CaCl₂ applications as a postharvest treatment differs

among crops (Shorter and Joyce, 1998). For green-harvested tomato, vacuum infiltration was found to be effective in rapid reduction of ethylene production and respiration rates at 20 °C, which was also dependent upon vacuum pressure and the concentration of CaCl₂ used (Wills *et al.*, 1977; Wills and Tirmazi, 1979). It was further reported that ripening could be delayed only when the calcium content of fruits was raised to a level greater than 40 mg/100g fresh weight (Wills *et al.*, 1977).

In the present study the effect of different modes of CaCl₂ application at different concentrations was examined for a local tomato variety 'Thilina' harvested at commercial maturity. The postharvest quality of fruits treated with CaCl₂ was evaluated under normal tropical storage conditions. The likely pathways of calcium were also studied using a water-soluble dye.

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MATERIALS AND METHODS

Plant material

Mature turning (1/10th orange) tomatoes (cv. 'Thilina') were collected from a commercial field at Kalugamuwa, Sri Lanka, (080° 35' longitude and 007° 15' latitude). Fruits visibly free from disease and defects were selected for the experiments. Fruits were packed into a three-ply corrugated fiber board box (CFB) with ventilation holes and lined with moulded pulp trays. Tomatoes were transported to the Plant Pathology Laboratory at the Department of Botany, University of Peradeniya, within one hour after harvest. Fruits were then rinsed with tap water and dipped in an aqueous solution of 1% sodium hypochlorite for 1 min for surface sterilization. Fruits were then allowed to drain for 30 min and used in the subsequent experiments.

Experiment 1: Mode of CaCl₂ treatment on the shelf life of tomatoes

Dip treatment with CaCl₂

Tomatoes were separated into 10 batches, each having 3 replicate fruits. Fruits were dipped separately in 3 different concentrations of calcium chloride (2%, 4% or 6%) for 10, 20 and 30 min. They were allowed to drain and thereafter held under ambient conditions (28 ± 2 °C and relative humidity $65 \pm 2\%$). Fruits dipped in distilled water (0% CaCl₂) for the same time durations served as controls.

Vacuum infiltration

Calcium chloride solutions of 2%, 4% and 6% concentrations were used. The three replicate fruits for each concentration were immersed in each solution of CaCl₂ and subjected to vacuum pressures of -10, -20 and -30 kPa for 2-3 minutes (Perera and Karunaratne, 2002). After release of the vacuum, the fruits were kept immersed in the same solution for a further 5 min (Wills and Tirmazi, 1979) in order to facilitate a rapid influx of CaCl₂ solution (Weerakoon *et al.*, 2006). The same procedure was followed for the control set of fruits using distilled water. Fruits were allowed to drain for 30 min upon removal from the dip, and stored in clean, dry plastic trays at room temperature (28 ± 2 °C) and relative humidity ($65 \pm 2\%$).

Pressure infiltration

Calcium chloride infiltration was performed by slightly modifying the method of Perera and Karunaratne (2002). CaCl₂ solutions of 2 %, 4 % and 6 % concentrations were prepared and the three replicate fruits for each concentration were

immersed in the respective solutions and pressures of 10, 20 and 30 kPa (Wills and Tirmazi, 1979) were applied for 2-3 min. After release of the pressure, fruits were kept immersed in the same solutions for a further 5 min. Thereafter, the fruits were stored as described above.

Shelf life of fruits

The shelf life of fruits was estimated as the number of days taken to reach the red ripe stage of the peel. The peel colour was rated visually using a self-prepared scale of 0-5 based on the U.S. Department of Agriculture classifications (Anon, 1975). The colour development scale for ripening was: 0 - green; 1 - turning; 2 - one third pink; 3 - two third pink; 4 - orange and 5 - red.

Experiment 2: Effect of vacuum infiltration with different concentrations of CaCl₂ on physicochemical changes in harvested tomatoes

Vacuum infiltration was found to be the only effective mode of treatment with CaCl₂ in extending the shelf life of tomato. Therefore, fruits were vacuum infiltrated with 2%, 4% and 6% CaCl₂ solutions under -20 kPa as described in Experiment 1. Thereafter, assessments were carried out for skin colour, total calcium content, rate of ethylene production, firmness, percentage titratable acidity (%TA), total soluble solids (TSS) content and pH.

Estimation of the total calcium content in tomato tissues

The amount of calcium in the fruit tissue was determined by slightly modifying the method of Shorter and Joyce (1998). Locular gel tissues were removed and tissues from the inner and outer pericarp regions were collected separately and dried in an oven at 80 °C until they reached a constant weight. The samples were then ground to a fine powder using mortar and pestle. Then, 0.5 g from each sample was ashed in a muffle furnace at 525 °C. The ash was dissolved in 5 ml of 20% HCl, filtered and made up to a volume of 50 ml with deionized water. Calcium was measured using flame emission spectrophotometer (Jenway-PFP 7, U.K.). Tissue Ca²⁺ concentration was expressed as mg Ca²⁺/g dry weight.

Rate of ethylene production

Ethylene production rates were measured by slightly modifying the method described by Hoeberichts *et al.* (2002). Individual tomatoes were incubated in air-tight bottles for 1 h. At the end of the incubation period, 1 ml gas samples were withdrawn from the headspace of the bottle

using an airtight syringe and injected in to a gas chromatograph. Quantification of ethylene was carried out on a gas chromatograph (Agilent 4890 D, USA) at an oven temperature of 75 °C on a 2.0 m (length) x 3 mm (internal diameter) column packed with porapak Q (Hewlett Packard Co., USA). The carrier gas was Helium at 25 ml min⁻¹ and the chromatograph was fitted with a flame ionization detector, which was set to 250 °C. The injector temperature was set to 125 °C and hydrogen gas pressure was set at 140 kPa. The air pressure was set at 200 kPa. Data was collected using the Agilent GC chemstation software package (Rev.A.08.03 - 847). The gas chromatograph was calibrated against 10.1 µl l⁻¹ standard ethylene (Scott Specialty Gases, USA). Ethylene production rate was expressed as µl kg⁻¹ h⁻¹.

Firmness of tomatoes

Fruit firmness was measured using a hand-held penetrometer (Forestry Suppliers Inc., U.K). One reading from each replicate fruit was taken from the middle, one third of the fruit, and the mean was calculated.

Percentage titratable acidity (% TA)

20 ml of juice from a fruit was squeezed and diluted up to 50 ml with distilled water and 10 ml aliquots were titrated with 0.1 M NaOH using phenolphthalein as the indicator (Wills and Ku, 2001).

Total soluble solids (TSS) content

Juice from tomatoes was squeezed and the TSS (Brix°) was determined using a hand held refractometer (Model, 121, Yagami International Ltd, Japan) (Wills and Ku, 2001).

pH

pH was measured using a flat ended digital pH meter for surfaces (Model TPS aqua V.1, Australia). Three readings were taken from each fruit by removing the peel (exocarp). Readings were taken 1 cm away from stem end, 1 cm away from blossom end and from the middle one third of the fruit, and the means were calculated (Weerakoon *et. al.*, 2006).

Experiment 3: Revealing the path of calcium penetration

Black ink (Hero, China) was added to 4% CaCl₂ solution. Vacuum infiltration of fruits was carried out at -20 kPa for 3 min in CaCl₂-ink solution. After 24 h, individual fruits were sectioned at 0, ¼ 1 and 24 h after infiltration for visual inspection of the path of dye and CaCl₂

penetration (modified method of Shorter and Joyce, 1998).

Experiment design and data analysis

All experiments were arranged in completely randomized design (CRD) as follows: Experiment 1: The factorial design was 2 factor (4 concentrations x 12 treatments), where the number of replicates (n) = 3. Data were subjected to analysis using a general analysis of variance (ANOVA) function. Experiment 2: The factorial design was (4 concentrations x 4 treatments), each treatment having 3 replicates and the data were subjected to one way ANOVA. Experiment 3: the factorial design was 2 factor (4 parameters i.e. firmness, pH, %TA and TSS with 4 concentrations) and data were analyzed using one way ANOVA.

RESULTS

Experiment 1: Effect of mode of CaCl₂ treatment on the shelf life of tomato (cv. 'Thilina')

Vacuum infiltration with 6% CaCl₂ was found to be the most effective method among all three modes of treatment tested for extending the shelf life of tomatoes (Table 1). The shelf life increased with higher vacuum pressure in both 4% and 6% CaCl₂ solutions. Although vacuum infiltration under -30 kPa with 6% CaCl₂ gave the longest shelf life, it resulted in oozing out of juice from the stem end scar which led to the growth of fungi at the red-ripe stage of tomato fruits (data not shown). Of the application methods, CaCl₂ dip treatment and pressure infiltration did not have an effect on the shelf life of tomatoes (Table 1). Therefore, evaluation of postharvest physico-chemical parameters was carried out only with fruits subjected to vacuum infiltrated at -20 kPa.

Experiment 2: Effect of vacuum infiltration with different concentrations of CaCl₂ on physicochemical changes in harvested tomatoes

Fruit Skin colour

Treatment with CaCl₂ concentrations of 4 % and 6 % at -20 kPa delayed colour development in tomato fruits compared to untreated fruits (Fig. 1). There was no difference in the pattern of change in skin colour in CaCl₂ treated fruits versus untreated fruits.

Total calcium content

The total calcium content in both inner and outer pericarp regions of fruits treated with CaCl_2 were significantly higher ($p = 0.05$) than those of untreated fruits (Table 2). The Ca^{+2} levels in tissues increased significantly with increasing levels of Ca^{+2} in the solution. Also, significantly higher levels of Ca^{+2} were detected in the inner pericarp region than in the outer pericarp of treated fruits

Ethylene production rate

CaCl_2 treated tomatoes produced ethylene at significantly lower levels than untreated fruits at the early stages of incubation (Fig. 2). Also, treatment with CaCl_2 delayed the time taken to reach ethylene climacteric in fruits. Furthermore, the magnitude of the climacteric peak was lowered to a certain extent by CaCl_2 treatment.

Fruit firmness

CaCl_2 treated fruits showed significantly higher firmness compared to untreated fruits at the end of the shelf life (red-ripe stage) (Fig. 3). The increase in firmness was found to have a positive relationship with the treated CaCl_2 concentration.

Total soluble solids (TSS) content, %TA and pH

The TSS content in fruits significantly increased with CaCl_2 treatment. However, there was no consistent relationship between CaCl_2 treatment and the TSS content of tomato fruits at the end of the shelf life (Table 3). Similarly, there was no consistent relationship between CaCl_2 treatment and %TA of tomato fruits (Table 3). However, with 6% CaCl_2 concentration a significant reduction in %TA was observed. Although it was expected that pH would increase with higher CaCl_2 levels according to %TA values, pH was reduced to insignificant levels with the increase of CaCl_2 concentration (Table 3).

Experiment 3: Path of calcium penetration

The ink added to the CaCl_2 solution had traveled fast, mainly through the stem end scar and after about 15 min it had almost reached the core of the fruit (Fig. 4). There were no visible signs of the CaCl_2 ink solution entering through other parts of the fruit skin.

Table 1. Shelf life of tomato fruits (cv. 'Thilina') treated with different concentrations of CaCl_2 using three modes of application (Storage conditions: $28 \pm 2^\circ\text{C}$ and $65 \pm 2\%$ RH)

Mode of CaCl_2 treatment	Treatment	Shelf life (days)*			
		0% CaCl_2	2% CaCl_2	4% CaCl_2	6% CaCl_2
Vacuum infiltration	-30 kPa	Damaged	5d	11b	14a
	-20 kPa	Damaged	5d	7c	10b
	-10 kPa	5d	5d	5d	5d
Pressure infiltration	30 kPa	Damaged	5d	6d	8c
	20 kPa	Damaged	5d	5d	6d
	10 kPa	5d	5d	5d	5d
Dipping	30 mins	5d	5d	5d	5d
	20 mins	5d	5d	5d	5d
	10 mins	5d	5d	5d	5d

* Time taken to reach fruit skin colour Index 5

Values are means of three replicates. Mean values followed by different letters are significantly different at $p = 0.05$

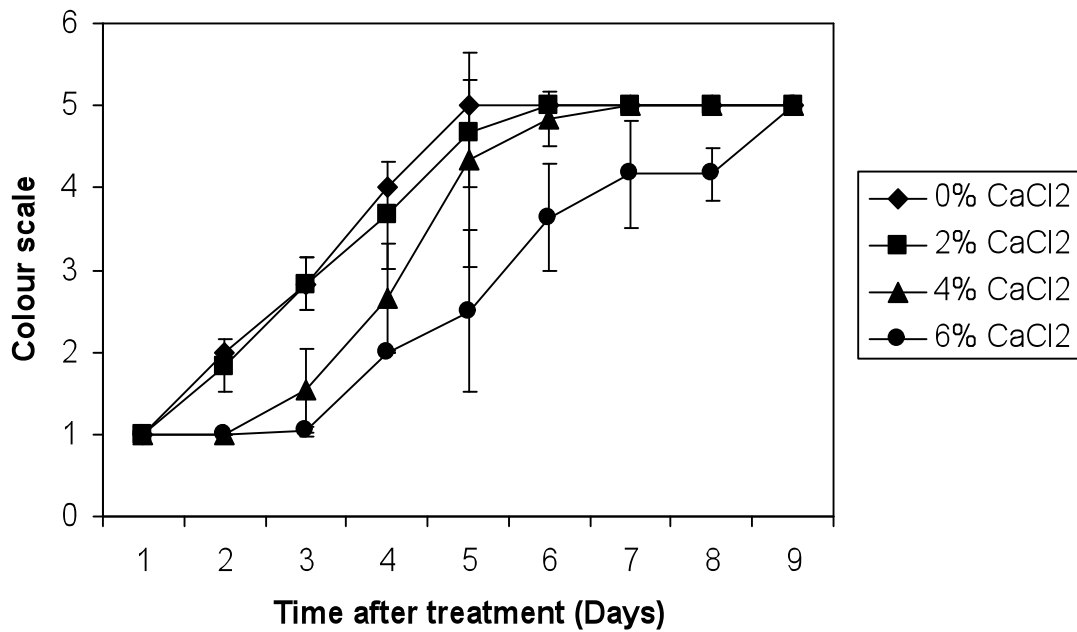


Figure 1. Effect of vacuum infiltration (at -20 kPa) with different concentrations of CaCl₂ on the skin colour change in tomatoes (cv. 'Thilina') stored under 28 ± 2 °C and 65 ± 2 % RH. (Vertical bars represent the standard error of mean) (Values are means of three replicates)

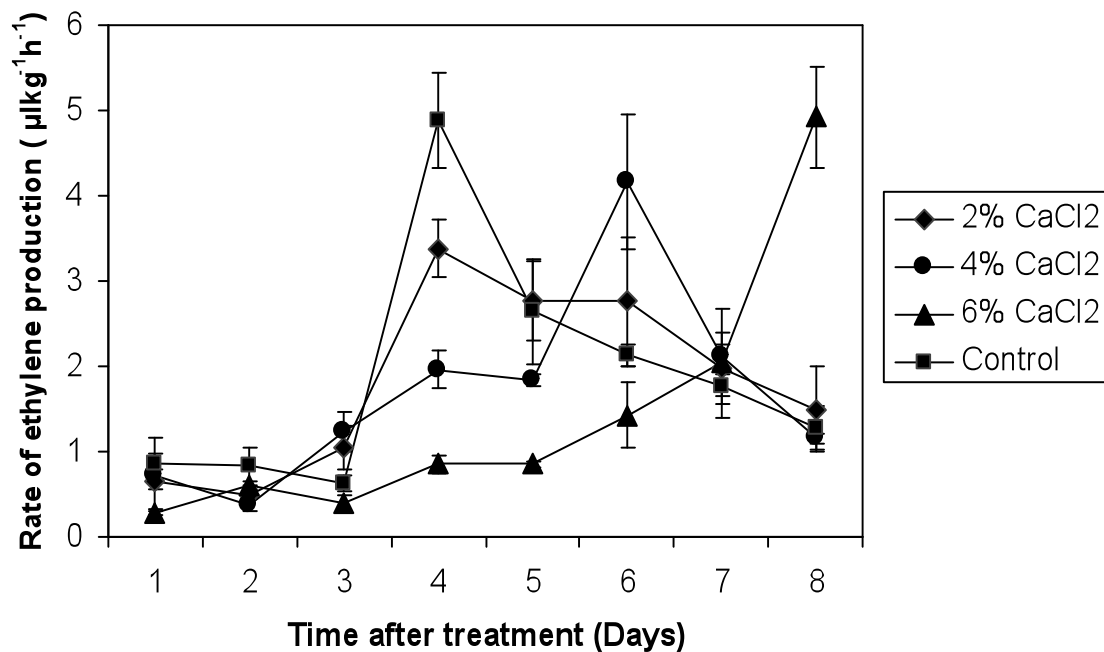


Figure 2. Effect of vacuum infiltration (at -20 kPa) with different concentrations of CaCl₂ on the rates of ethylene production in tomato (cv. 'Thilina') (Vertical bars represent the standard error of mean) (Values are means of three replicates)

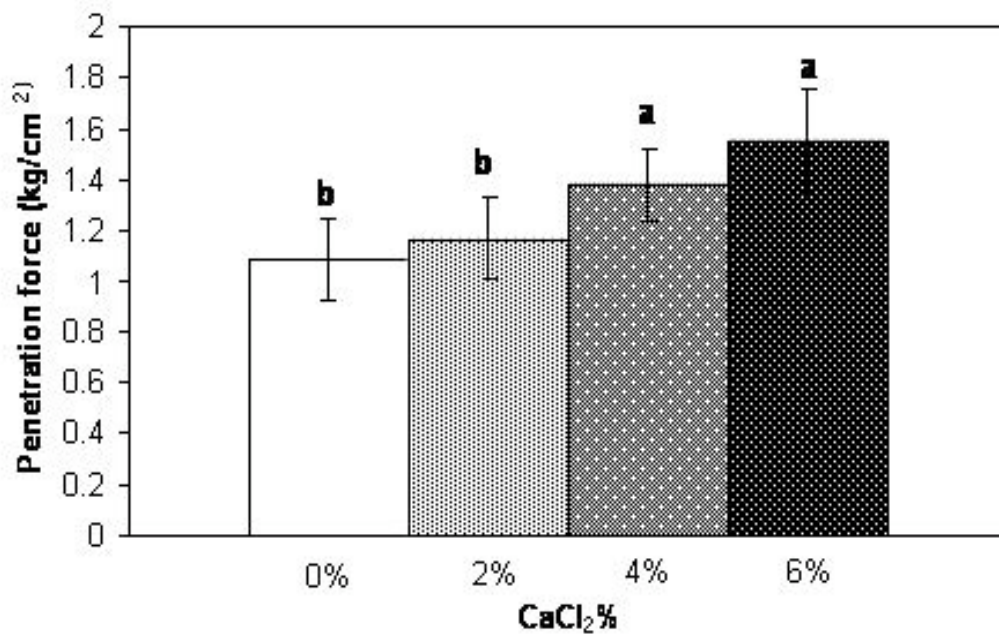


Figure 3. Effect of vacuum infiltration (at -20 kPa) with different concentrations of CaCl_2 on the firmness in tomatoes (cv. 'Thilina') at red-ripe stage (Colour Index 5).

(Vertical bars represent the standard error of mean)

(Values in each column followed by different letters are significantly different at $p = 0.05$)

(Values are the means of three replicates)

Table 2. The Ca^{2+} content in the inner and outer pericarp tissues of tomatoes (cv. 'Thilina') after vacuum infiltration with different concentrations of CaCl_2 for 2 min under -20 kPa.

% CaCl_2	Ca^{2+} content (mg/g dry weight)	
	Outer pericarp	Inner pericarp
0	20.7f	28.8c
2	22.8f	31.3b
4	24.3f	35.8b
6	27.5c	43.8a

Values are means of three replicates.

Mean values in each column followed by different letters are significantly different at $p = 0.05$



Figure 4. Longitudinal sections (L.S.) and Transverse sections (T.S.) of tomato fruit at different time intervals after vacuum infiltration (at -20 kPa for 2 minutes) with 4% CaCl_2 solution containing black ink.

Table 3. Total soluble solids content, percentage titratable acidity and pH of tomatoes (cv. 'Thilina') infiltrated with different concentrations of CaCl_2 at -20 kPa vacuum pressure and stored at $28 \pm 0^\circ\text{C}$ until red-ripe.

% CaCl_2	Total Soluble Solids (TSS) content	Percentage titratable acidity (TA %)	pH
0	3.30b	7.07a	4.55a
2	3.87a	7.58a	4.51a
4	3.80a	7.04a	4.48b
6	3.20b	6.59b	4.49a

Values are means of three replicates.

Mean values in each column followed by different letters are significantly different at $p = 0.05$

DISCUSSION

The technique of vacuum infiltration has been reported to be effective in raising the calcium content in fruits like mango (Tirmazi and Wills, 1981; Shorter and Joyce, 1998), avocado (Tingwa and Young, 1974), tomato (Wills *et al.*, 1977, Wills and Tirmazi, 1979) and pears (Wills *et al.*, 1982). Higher concentrations viz. 12 % CaCl_2 may result in complete inhibition of ripening as shown by the inhibition of fruit colour development in certain fruits (Wills *et al.*, 1977). Reasons for the failure in skin colour development may be an effect of CaCl_2 on the ethylene generating cycle, which affects the synthesis of the pigment lycopene during the process of ripening (Njoroge *et al.*, 1998). The change in tomato fruit colour appears to be dependent also upon the vacuum applied, maturity of fruits and the storage temperature (Moline, 1980).

The total Ca^{2+} in tomato tissue showed a positive relationship with the CaCl_2 concentrations used for infiltration. There was a significant increase in Ca^{2+} in the inner pericarp and a lesser increase in the outer pericarpal region. As revealed by the dye penetration test, most of the treatment solutions had entered through the stem end scar. This may have led to a greater accumulation of calcium in the inner pericarpal region. The amount of Ca^{2+} in the outer pericarp was relatively low, which may possibly have entered through the stomata. Wills *et al.* (1977) have reported that a 3-fold increase in calcium level (compared to untreated fruits) is needed to produce any noticeable retardation in the ripening of green tomato (cv. 'Rouge de Mamonde') at 20 °C. However, in the present study, even a 1.4 fold increase in Ca^{2+} levels (compared to the control) was effective in retarding ripening. These observed differences in different studies may be due to the relatively higher storage temperatures (27 ± 2 °C), cultivar difference and also the differences in the stage of fruit maturity at the time of CaCl_2 treatment.

The time taken to reach the ethylene climacteric was also delayed with the increase in CaCl_2 concentration. It has been shown that once the intracellular calcium concentration increased to at least 1 μM (Saunders and Helper, 1983), calcium is bound to calmodulin, which is one of the most common intracellular calcium receptors and the accruing calcium-calmodulin complex modulates many physiological processes. Njoroge *et al.* (1998) found that calmodulin is involved in the calcium inhibited ethylene

biosynthesis. Calcium may be inactivating the Ethylene Forming Enzyme in the ethylene biosynthetic pathway via calcium-calmodulin mediated reactions.

There was a positive relationship between tomato fruit firmness and the treated CaCl_2 concentration. The stability of the cell walls may probably be attributed to the formation of cation cross bridges between uronic acid groups (Sams *et al.*, 1993) as evident from increased firmness levels in apples (Wienke, 1980). With 6% CaCl_2 treatment, the higher levels of calcium accumulated in the tomato pericarp may have contributed to significantly higher fruit firmness through such cross-linking. Generally, calcium is found in the cell walls as calcium pectate (Agusti *et al.*, 2004). Vacuum infiltration of calcium may also have increased the bound calcium content in the tomato tissues contributing to an increase in the fruit firmness (Hong and Lee, 1999). The higher firmness at eating-ripe stage of tomato may be attributed to the inhibited action of polygalacturonase (Doesburg, 1975), the enzyme which mediates the degradation of pectates during ripening.

Calcium treatment has little effect on TSS content, pH or TA in tomato fruits. This observation agrees with reports on fruits like mango (Tirmazi and Wills, 1981) and strawberry (De Souza *et al.*, 1999). However, the effect of CaCl_2 treatment on chemical parameters appear to be commodity-dependent.

In conclusion, application of 6% CaCl_2 at -20 kPa vacuum pressure could be recommended as a commercial postharvest treatment for tomato under tropical storage conditions. Extension of the shelf life may be attributed mainly to the increased firmness and retarded ethylene production rates in CaCl_2 treated fruits. Furthermore, evaluation of sensory parameters with a trained taste panel may be useful to confirm the acceptability of CaCl_2 treatment in terms of flavour quality of tomatoes.

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